Armed Forces College of Medicine

AFCM

Hormone action and signal transduction

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INTENDED LEARNING OBJECTIVES (ILO)

By the end of this lecture the student will be able to:

1. **Interpret role of calcium as a mediator of hormone action.**
2. **Explain mechanism of action of hormones using cAMP as second messenger.**
3. **Correlate disruption in hormone signaling to clinical disorders**

Hydrophilic Hormones

They have different second messengers:

1- cAMP

2- cGMP

3- Calcium and /or Phosphatidyl inositol

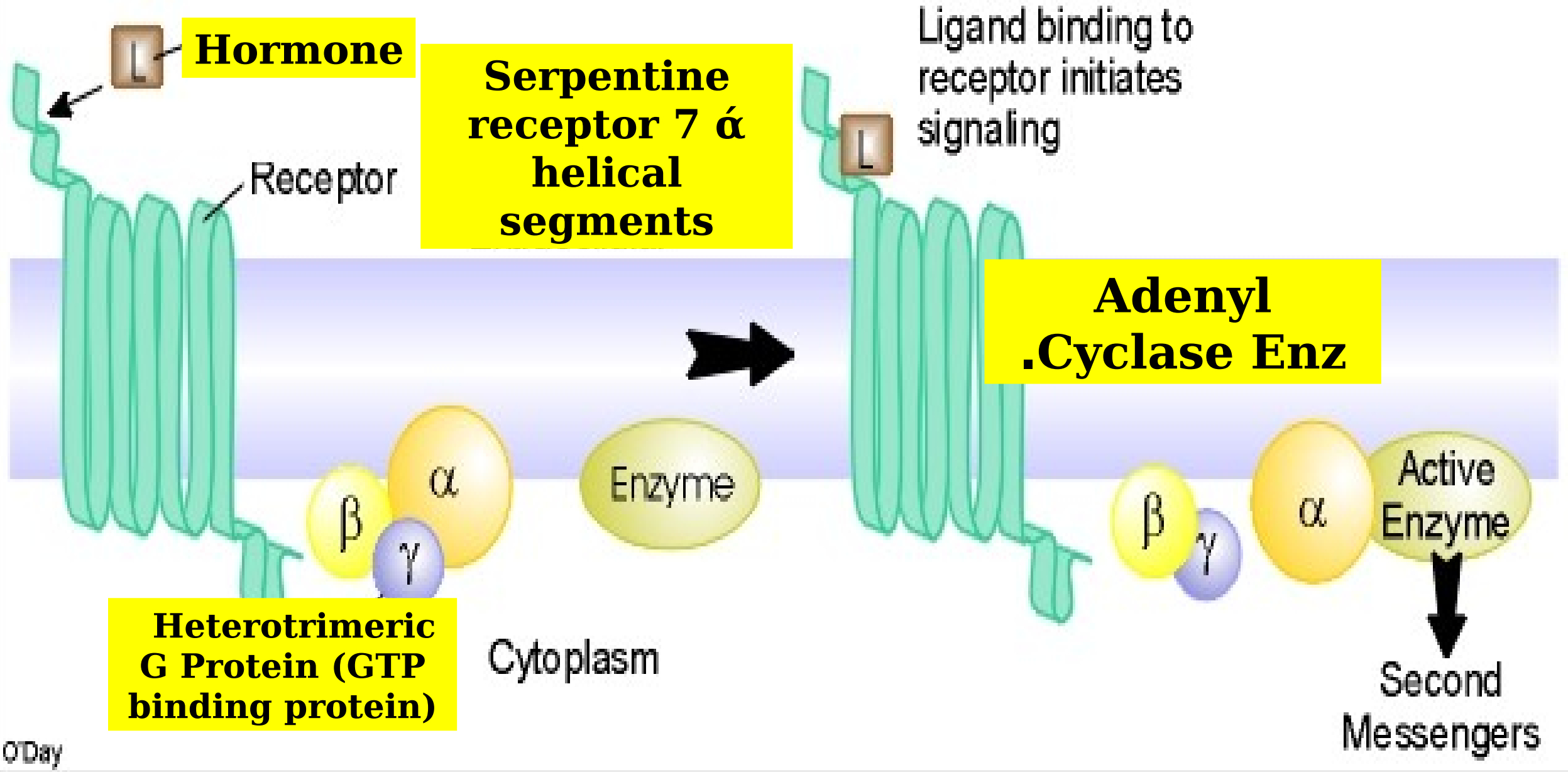
4- Kinases

1-cAMP as a second messenger

Hormones acting by this method:

- **Glucagon,**
- **β adrenergic catecholamines,**
- **FSH, LH, ACTH, TSH**

G Protein- G-protein coupled adenyl cyclase-cAMP system



G protein signaling

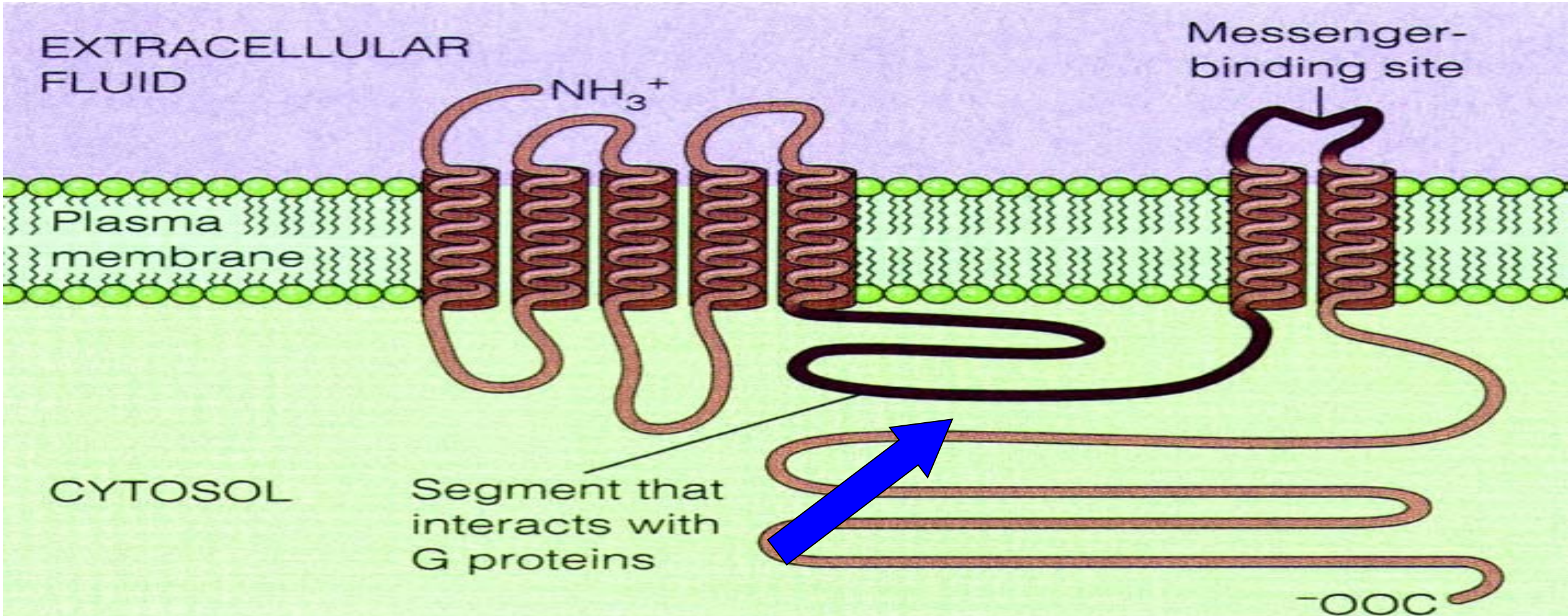
G proteins:

Intracellular signaling proteins

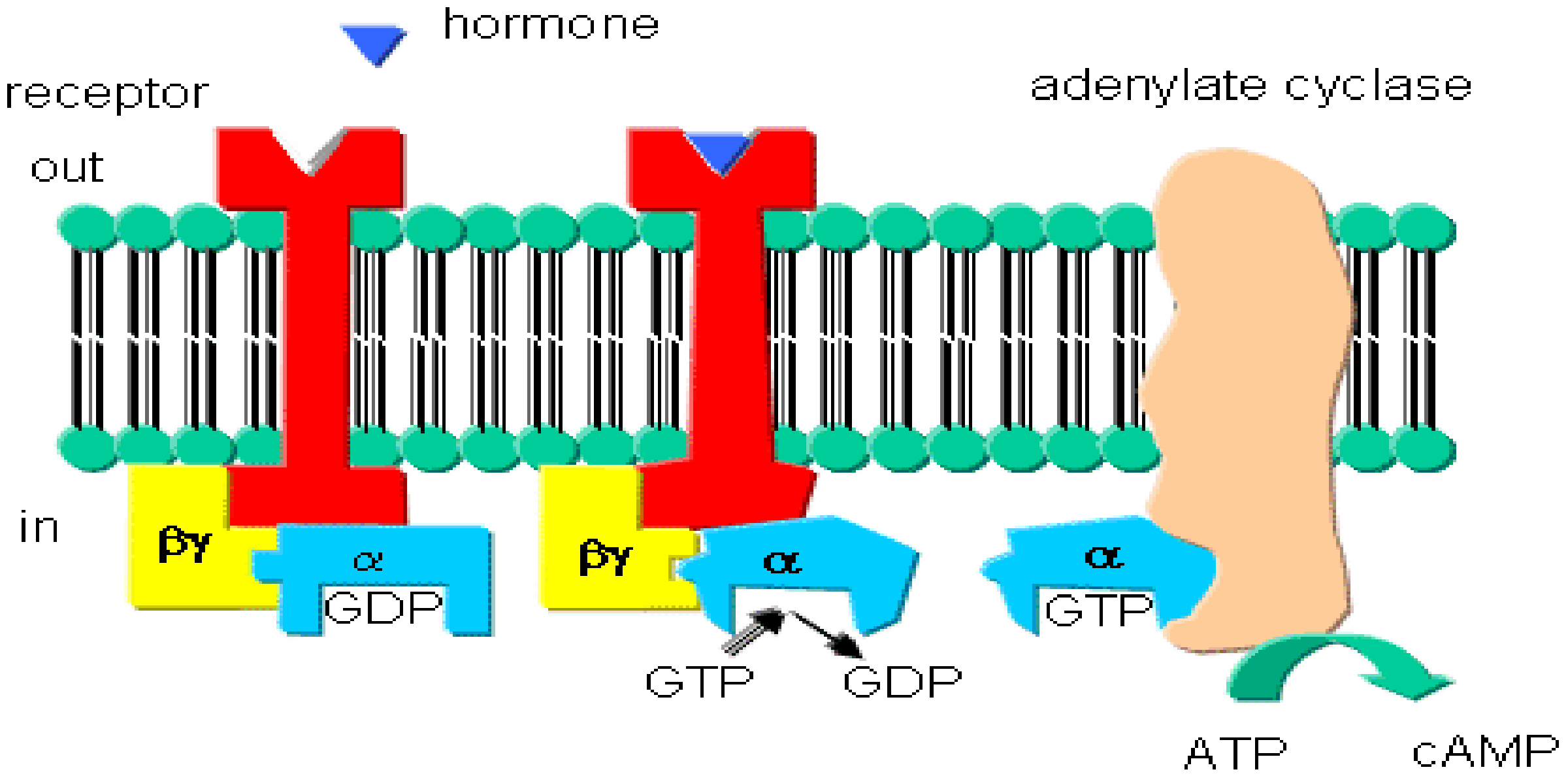
Named for their binding to GTP

Have GTPase activity; can hydrolyze GTP to GDP.

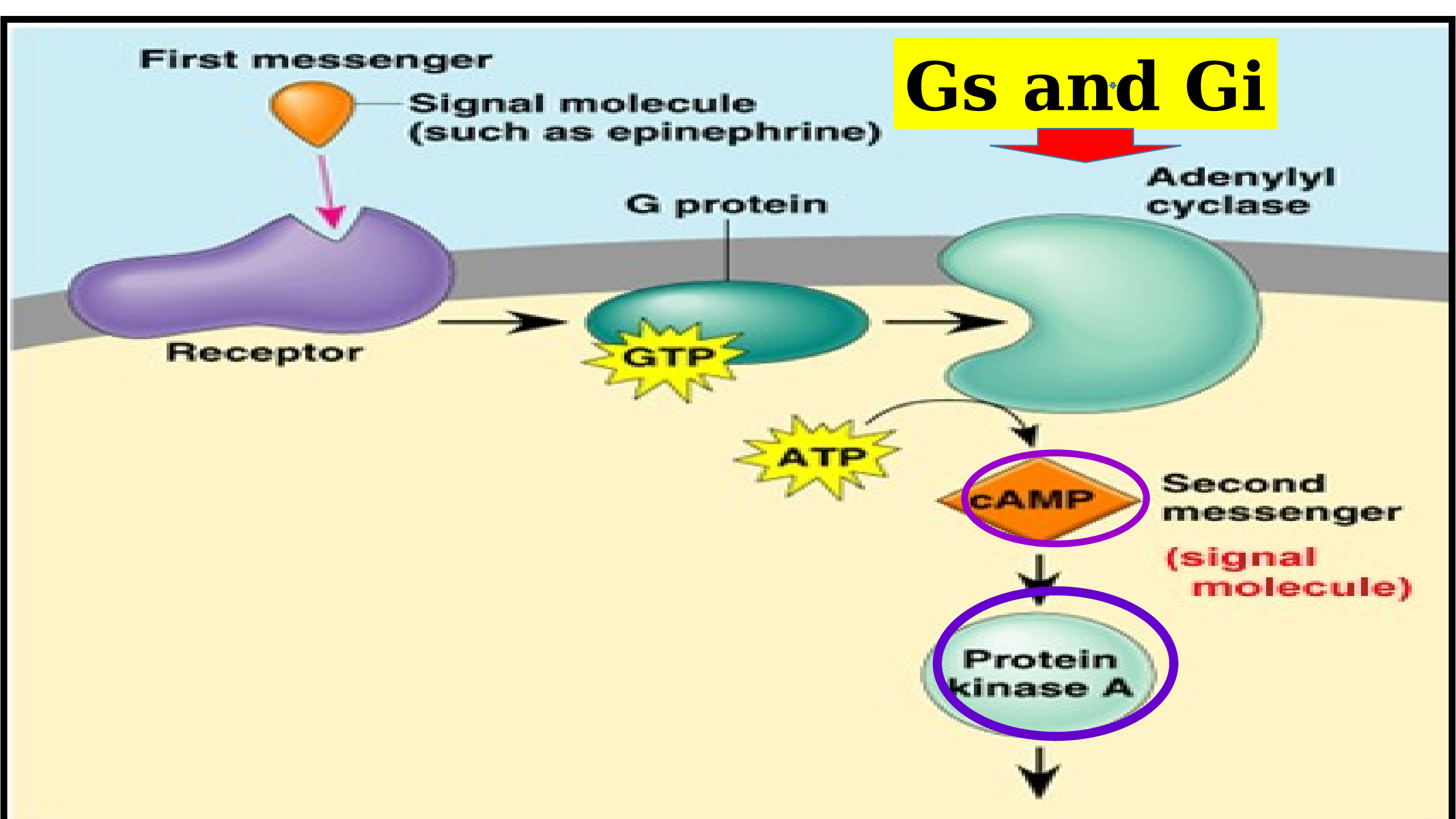
Serpentine Receptors



G protein activation of adenylate cyclase



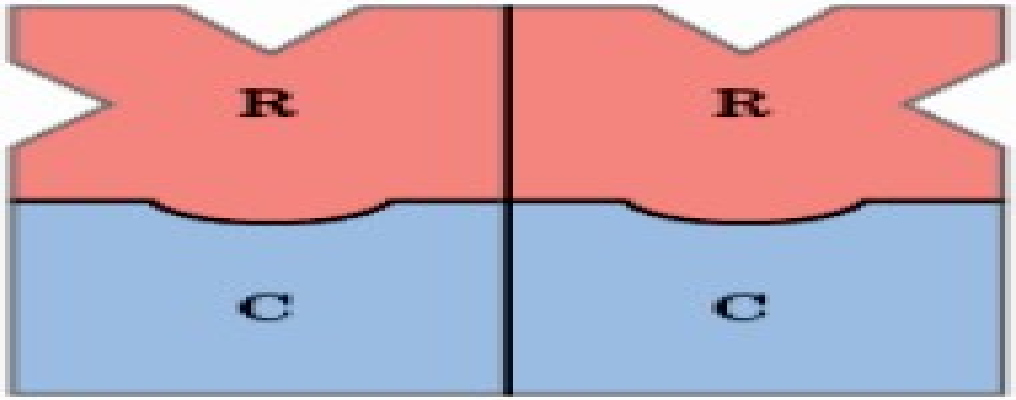
Gα subunit	Action
α_s; Gα (s) *	stimulates adenyl cyclase
$\alpha_{i/o}$; Gα (i,	inhibits adenylyl cyclase
$\alpha_{q/11}$; Gα (q/	activates phospholipase C_β


Gs and Gi
First messenger
Signal molecule
(such as epinephrine)
Adenylyl
cyclase
G protein
Receptor
GTP
ATP
cAMP
Second
messenger
(signal
molecule)
Protein
kinase A

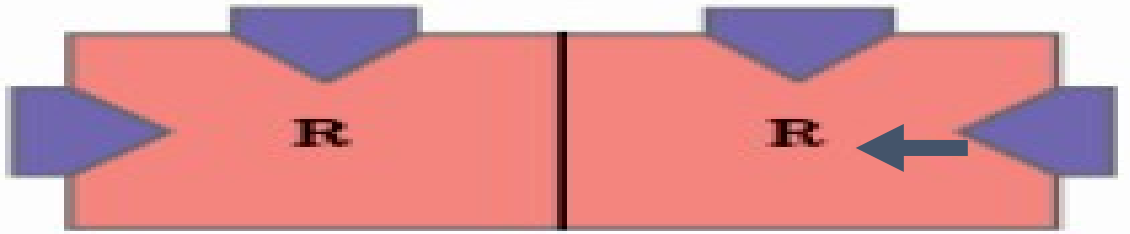
Inactive PKA

Regulatory subunits:
empty cAMP sites

Catalytic subunits:
substrate-binding
sites blocked by
autoinhibitory
domains of R subunits



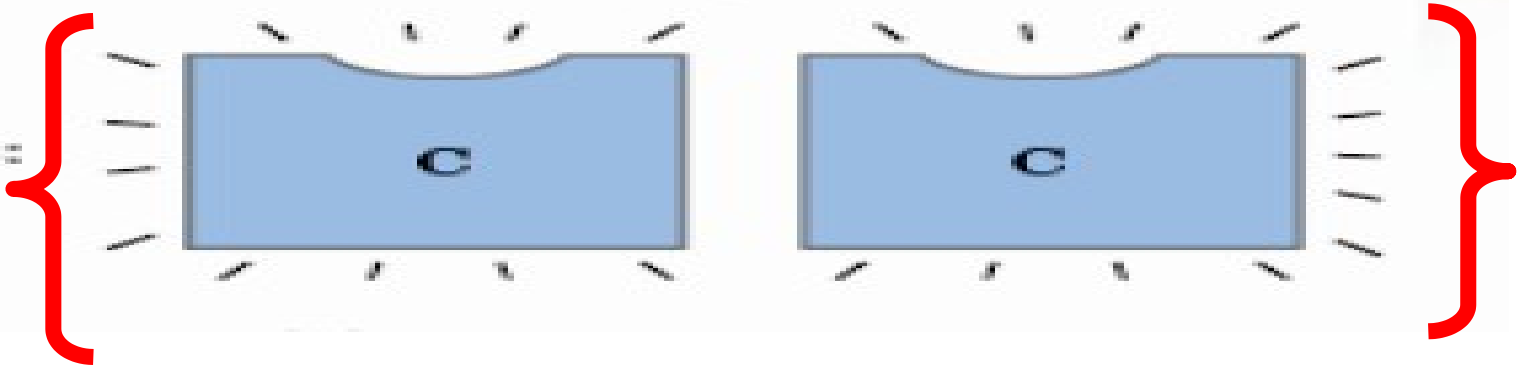
Regulatory subunits:
autoinhibitory
domains buried



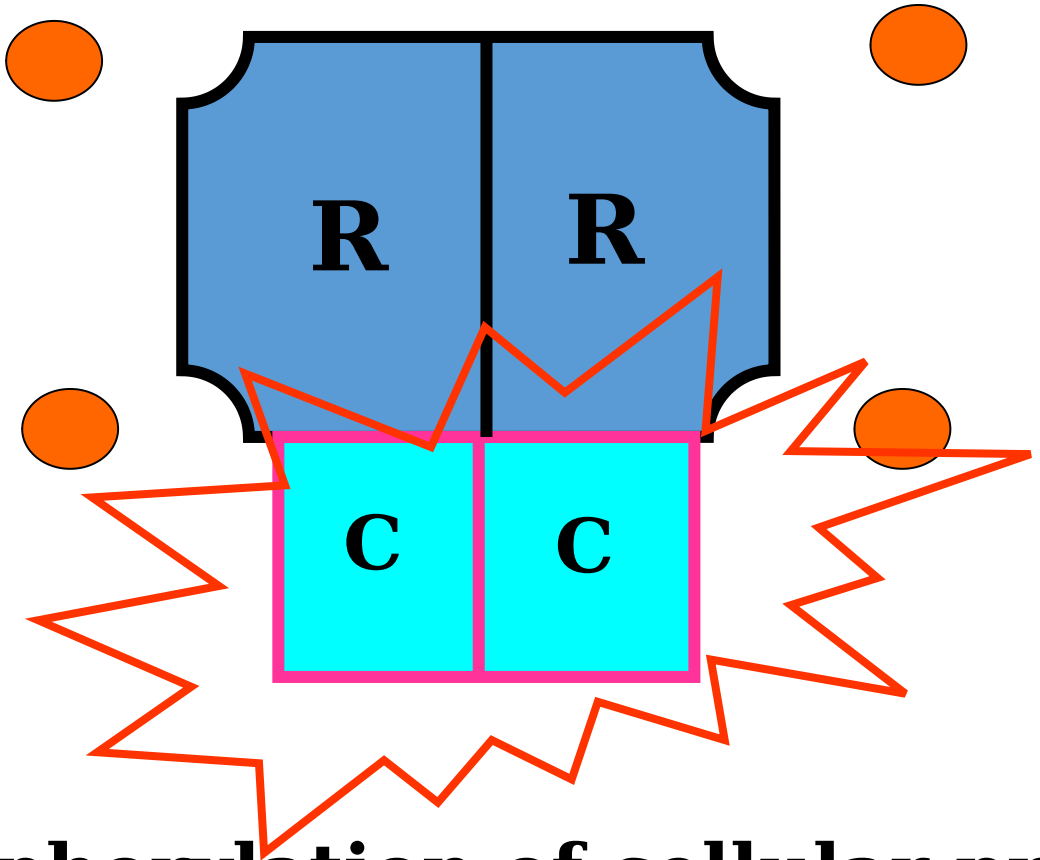
+

Active PKA

Catalytic subunits:
open substrate-
binding sites



cAMP dependant Protein kinase Enzyme



Phosphorylation of cellular proteins at serine or threonine AA .That stimulate or inhibit some enz

Phosphprylation can stimulate or inhibit some enzymes

1- Glycogen synthase (synthesis of glycogen) active in dephosphorylated form.

2- Glycogen phosphorylase (degradation of glycogen) active in phosphorylated form.

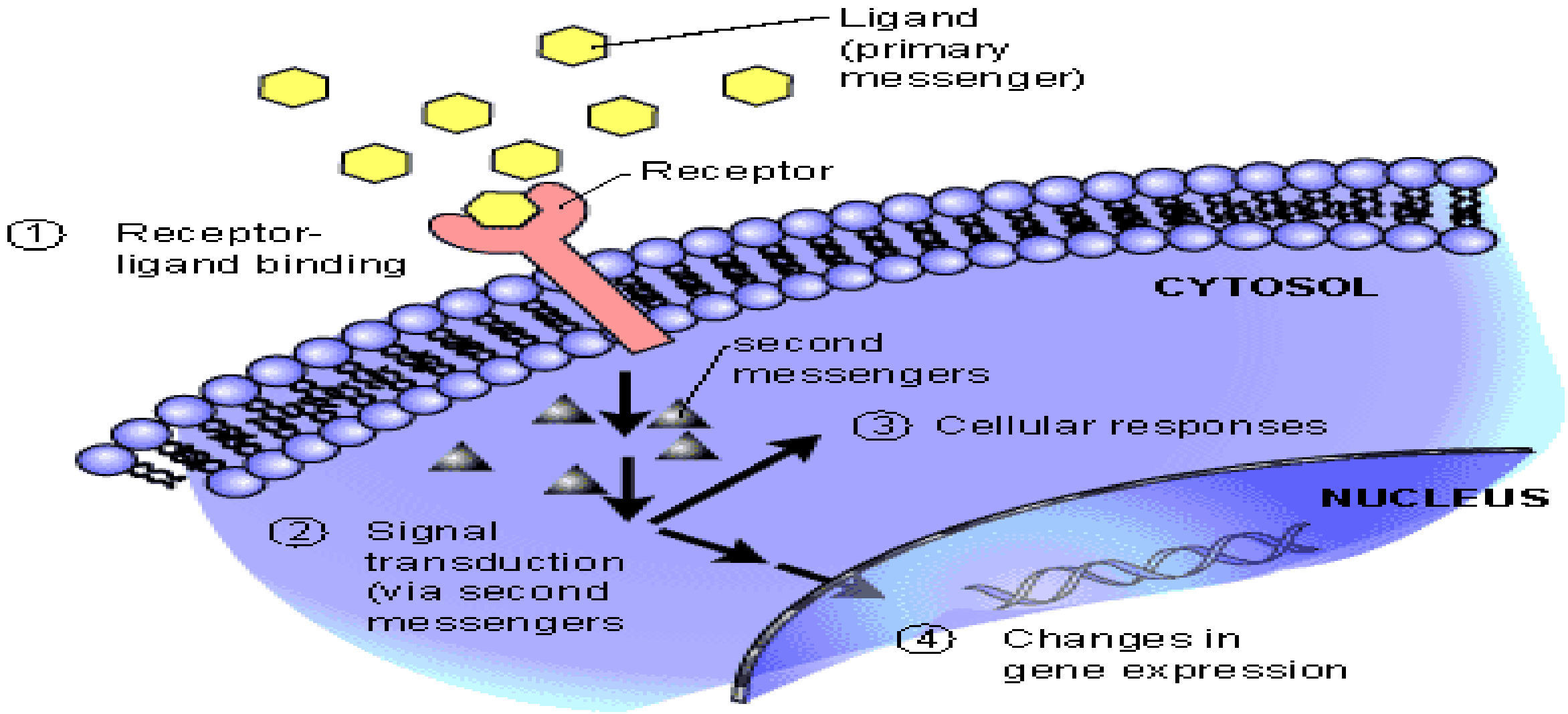
G Protein signaling

- **The G protein, stimulated by the activated receptor, exchanges bound GDP for GTP on α subunit with concomitant dissociation of βγ from α.**
- **The active G-protein dissociates from the occupied receptor and the GTP-loaded Gα binds to the effector enzyme, adenyl cyclase, activating it. This stimulatory G-protein is termed Gs.**
- **Gα subunits are distinguished from each other by subscripts including s, i, and q (Gαs, Gαi, and Gαq), Gs stimulates adenylate cyclase enzyme while Gi inhibits it. Gq stimulates phospholipase C.**

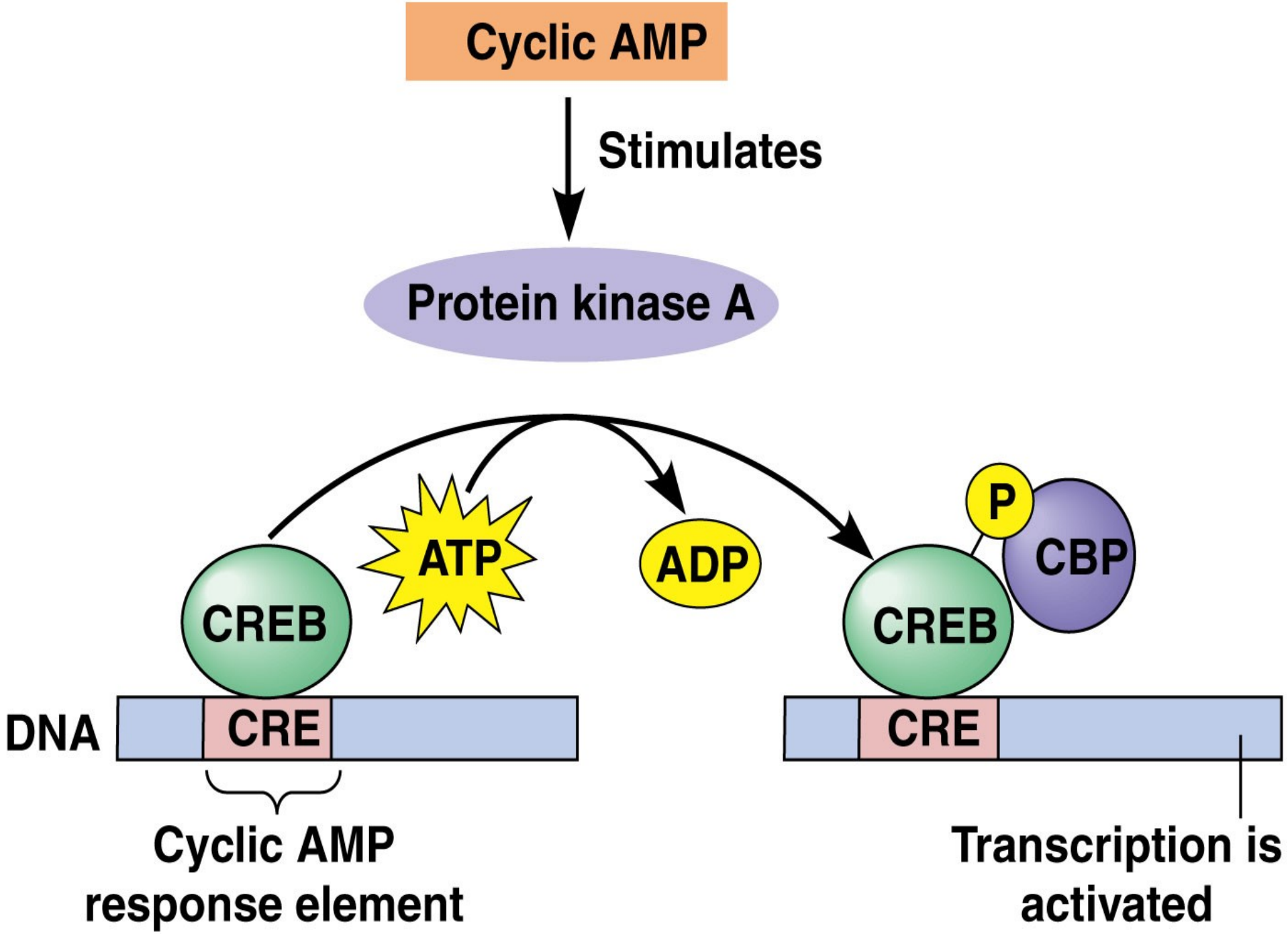
G Protein signaling

- **<u>Adenyl cyclase (AC)</u> is an integral protein of the plasma membrane, It catalyzes the synthesis of cAMP from ATP.**
- **Cyclic AMP binds to protein kinase A (PKA) (cAMP-dependent protein kinase) and activates it. The inactive form of PKA contains two catalytic subunits (C) and two regulatory subunits (R).**
- **The tetrameric R2C2 complex is catalytically inactive, because each R subunit occupies the substrate-binding site of one C subunit.**
- **When cAMP binds to two sites on each R subunit, the R subunits undergo a conformational change and the R2CA complex dissociates to yield two free (C) subunits.**
- **These catalytic subunits are able to phosphorylate target**

Effect of cAMP on transcription



cAMP response element binding protein CREB



Effect of cAMP on transcription

Rise in cAMP

↓

cAMP response element-binding protein (*CREB*) is *phosphorylated* and *activated*

↓

Active CREB binds the coactivator CBP (CREB-binding protein)

↓

Active CREB binds the cAMP-response element (*CRE*)

↓

***Transcription* of target genes with CREs in their promoters**

This Hormonal action is terminated:

1-GTPase activity of ά subunit that converts GTP into GDP with

re-association of the three subunits to return to the resting state.

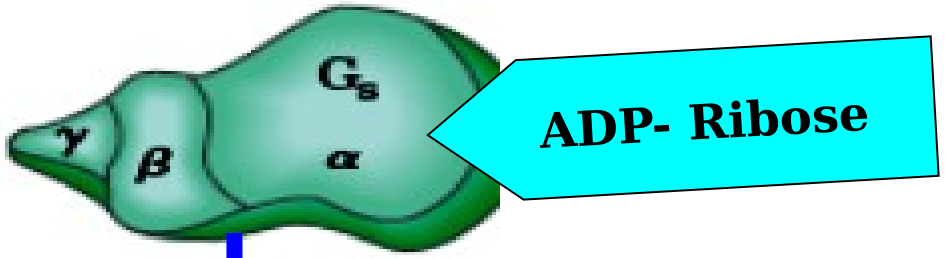
This Hormonal action is terminated:

2- *Phosphodiesterase* that convert cAMP into 5-AMP.

3-*Phosphatases* remove phosphate from phosphorylated proteins and thus terminate the hormonal action.

Toxins disrupt G protein

Cholera toxins **are enzymes catalyze ADP ribosylation of ά subunit of G_s Of intestinal cells**



Blocking GTPase activity

Continuous activation of Adenyl cyclase of intestinal cells

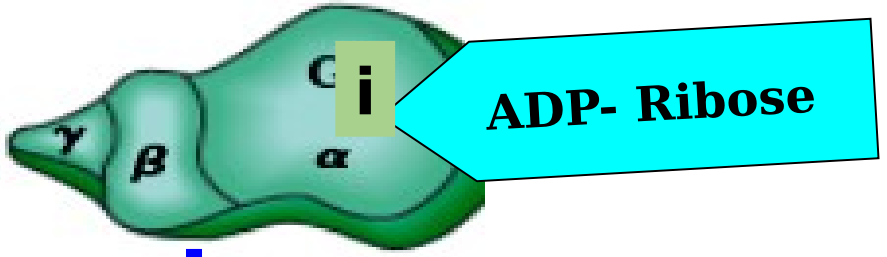
↑ cAMP

Continuous secretion of CL^- , HCO_3 and water

Diarrhea & dehydration

Toxins disrupt G protein

Pertussis toxins **secreted by Bordetella pertussis are enzymes catalyze ADP ribosylation of ά subunit of G_i**



Preventing displacement of GDP by GTP and blocking inhibition of adenyl cyclase by G_i

cAMP

Whooping cough symptoms

Disruption of G-Protein signaling causes disease

- Cholera toxin, an enzyme secreted by vibrio cholerae found in contaminated drinking water, catalyzes the transfer of ADP-ribose from NAD to the α-subunit of Gs, blocking its GTPase activity and thereby rendering Gs permanently activated.
- This results in continuous activation of the adenyl cyclase of intestinal epithelial cells and chronically high cAMP, which triggers constant secretion of Cl, HCO3 and water into the intestinal lumen resulting in dehydration and electrolyte loss.
- Pertussis toxin, an enzyme produced by Bordetella pertussis, catalyzes ADP ribosylation of Gi, preventing displacement of GDP by GTP and blocking inhibition of adenyl cyclase by Gi. This defect produces 2 of whooping cough symptoms.

In cholera, there is uncontrolled secretion of sodium ions and water into the intestinal lumen because of the action of cholera toxin on a G protein coupled receptor system. How does the toxin act?

a) Cholera toxin activates a Gi (inhibitory) protein.

b) Cholera toxin inhibits phosphodiesterase so that the signal is not switched off.

c) Cholera toxin inhibits the binding of vasoactive intestinal polypeptide to the receptor.

d) Cholera toxin inhibits the GTPase activity of the G protein alpha subunit.

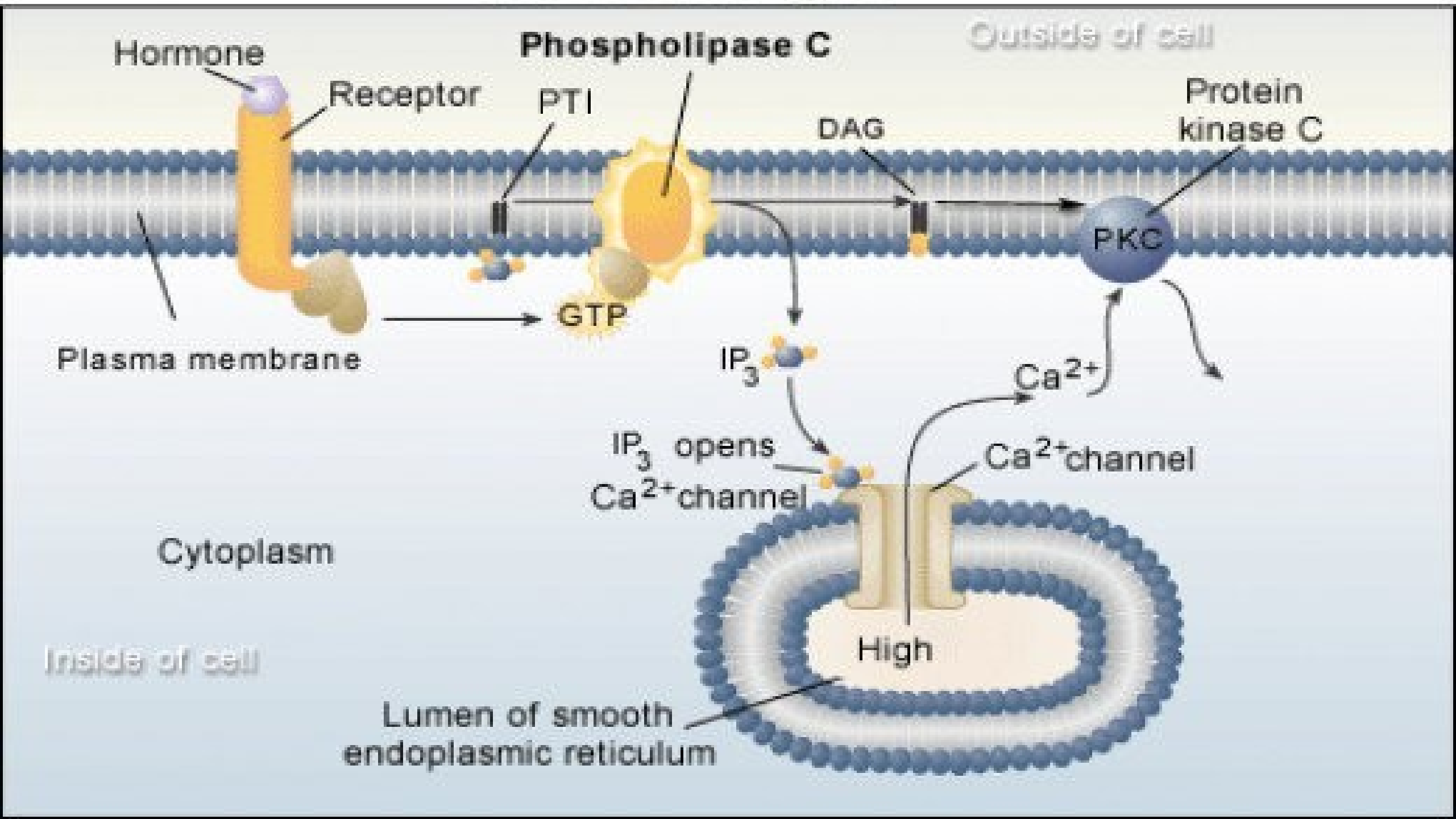
2-Calcium and /or Phosphatidyl inositol as second messengers

Examples of Hormones acting by this method:

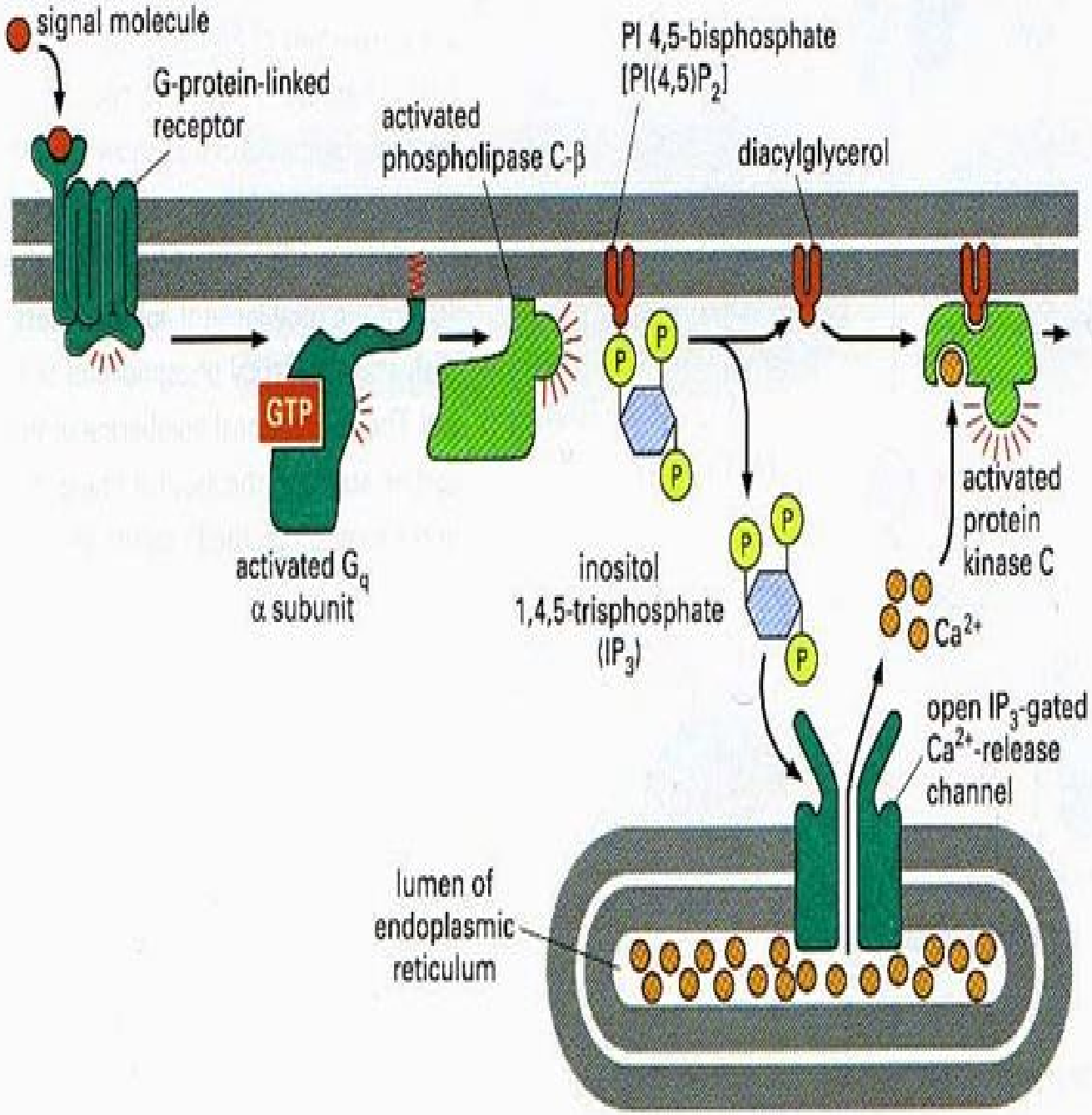
***α_1 adrenergic catecholamines,**

*** vasopressin,**

*** oxytocin**


Hormone
Receptor
Phospholipase C
PTI
Outside of cell
Protein kinase C
DAG
PKC
GTP
Plasma membrane
IP3
Ca2+
IP3 opens Ca2+ channel
Ca2+ channel
Cytoplasm
Inside of cell
High
Lumen of smooth endoplasmic reticulum

Normally cytosolic Ca^{2+} is kept very low by its pumping inside mitochondria and ER by Ca^{2+} pumps.



2-Calcium /DAG/ IP3 signaling:

1. A hormone binds its specific **serpentine receptor** in the plasma membrane

2. The receptor-hormone complex catalyzes **GTP-GDP exchange** on a G protein, **Gq**.

3. The **α-subunit** of activated Gq **dissociates** from βγ and **activates PLC**

4. **PLC** hydrolyzes **phosphatidylinositol 4,5-bisphosphate** (PIP2) in the plasma membrane into **DAG** (diacylglycerol) and **IP3** (inositol 1,4,5-triphosphate) which act as **second messengers**.

2-Calcium /DAG/ IP3 signaling:

5. Ip3 diffuses from plasma membrane to the ER

6. IP3 binds to IP3-gated calcium channel receptors within ER.

7. Opening of calcium channels occur

8. Release of sequestrated calcium into the cytosol

9. Rising of cytosolic calcium level

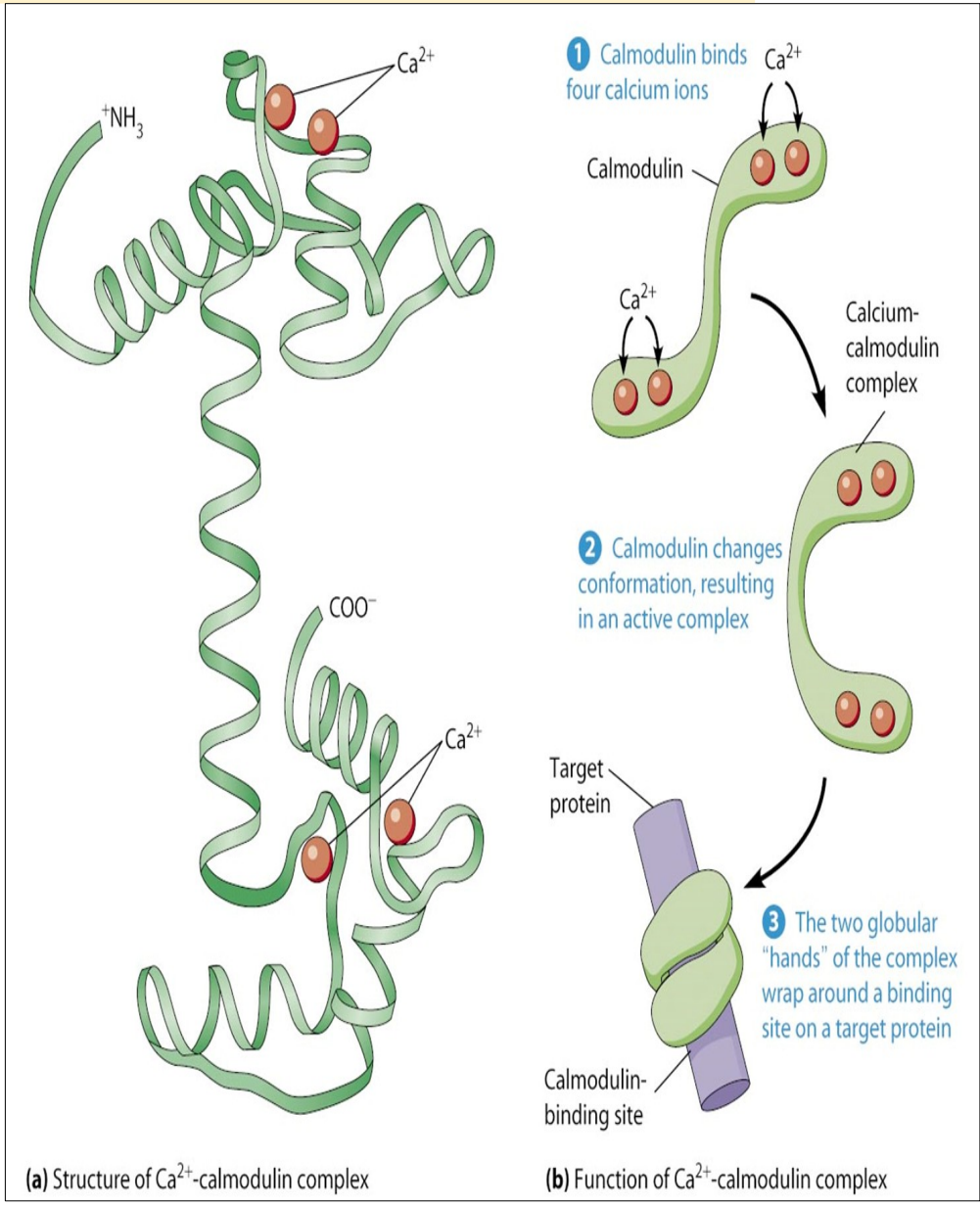
Role of Calcium as a second messenger:

Its affects target proteins by:

1-Directly activating certain enz. e.g protein kinase C (PKC)

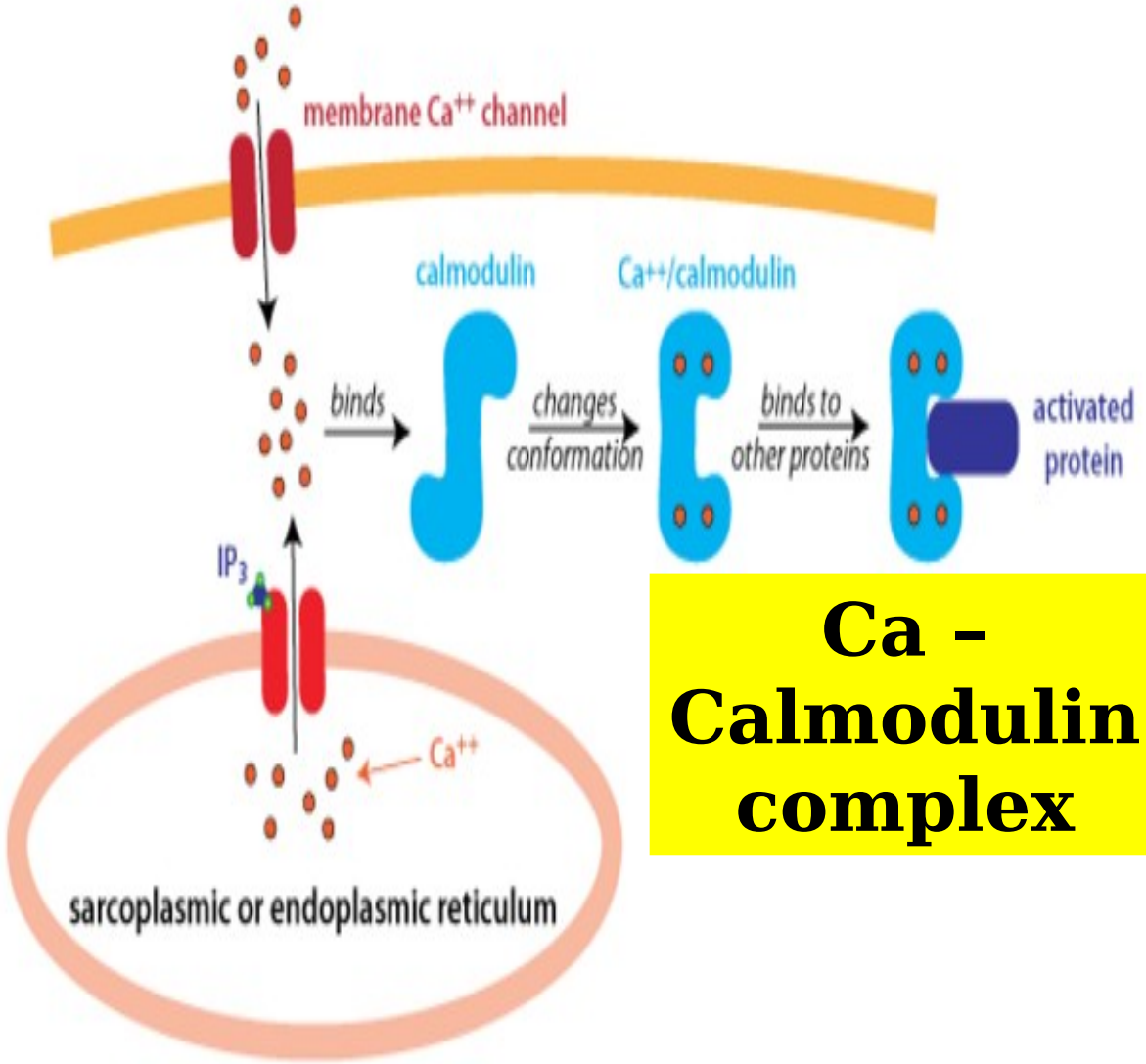
N.B: ***DAG is hydrophobic and so it remains in the membrane and cooperates with Ca in activating PKC.***

2-Indirectly through calmodulin (regulatory protein)



Role of Calcium as a second messenger:

- **Calmodulin is a regulatory protein. It has 4 binding sites for Ca.**
- **When Ca occupies its 4 binding sites, calmodulin exhibits a conformational change that activates the kinases.**
- **The kinases then phosphorylate a number of target enzymes, modifying**



Ca – Calmodulin complex

Thank you